transplant protocols such as the type of GVHD prophylaxis used. Nevertheless, the skin explant model remains a unique *in vitro* system which provides an *in situ* histopathological read-out for studying alloreactivity and human GVHD. The model has also the potential to aid the development of novel prophylaxis and treatment for GVHD.

170

INTERFERON-α THERAPY FOR PATIENTS WITH MOLECULAR RELAPSE IN LEUKEMIA AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

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One of the problems in allogeneic stem cell transplantation (alloSCT) for acute leukemia (AL) is the recurrence of the disease. Wilms' tumor gene (WT1) expression has been reported to be expressed in leukemic cells and used as a prediction marker of leukemia relapse after chemotherapy (CT) in patients with AL. Recently, Ogawa et al. described that the quantitative measurement of WT1 expression in bone marrow cells (BM cells) assisted the prediction of leukemia recurrence after allo-SCT (*Blood* 2003). Several reports showed that interferon α (IFNα) administration was effective in the prevention of leukemia recurrence in patients after alloSCT. Based on this evidence, we tried to prevent the recurrence of AL by IFNα administration in patients with alloSCT. We reported here three patients with molecular relapsed AL after alloSCT who received the administration of IFNα to get and maintain complete remission (CR). The molecular relapse was defined as the criteria, described by Ogawa et al. They could not receive donor lymphocyte infusion (DLI) because of no acceptance of donor. Case 1 (SI): The patient with refractory AML received BMT from unrelated donor (uBMT). He began to receive IFNα (2 MU/m^2 , four times per week) on day 84, when the molecular relapse was diagnosed, and maintained the administration for 1 month. WT1 expression in BM cells decreased rapidly (3200 → 82 copies/mg RNA). He has achieved CR more than 30 months. Case 2 (TM): The patient with high risk AML received PBSCT from sibling donor. She began to receive IFNα (2 MU/m^2 , two times per week) on day 384, when the molecular relapse was diagnosed, and had kept the administrations for 4 months. WT1 expression in BM cells decreased rapidly (4100 → 1100 copies/mg RNA). She has maintained CR for 21 months. Case 3 (HK): The patient with high risk AML received uBMT. He began to receive IFNα (2 MU/m^2 , two times per week) on day 209, when the molecular relapse was diagnosed, and had kept the administration for 2 months. WT1 expression in BM cells decreased rapidly (11000 → 1100 copies/mg RNA). He had maintained CR for 12 months. His WT1 expression level re-increased on day 366, and a BM aspirate showed 18.5% leukemic cells on day 387. He again received the CT and alloSCT. In each case, WT1 expression in BM cells decreased rapidly. They could keep CR more than 5 months (30, 21 and 5 months). These results strongly suggest that IFNα might be useful in treating molecular relapsed leukemia after alloSCT.

171

IMPORTANCE OF DETERMINING TRANSFORMING GROWTH FACTOR β1 PROMOTER ALLELES TO PREDICT EXPRESSION PHENOTYPE AND ITS POTENTIAL IMPACT ON BONE MARROW TRANSPLANTATION

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Transforming growth factor beta 1 (TGF-β1) is a multifunctional cytokine that plays a crucial role in immune regulation. Three of eight known polymorphic sites in the human TGF-β1 5′ regulatory and signal peptide regions have been associated with higher secreted levels of TGFβ1. These single nucleotide polymorphisms (SNPs) have been linked to bone marrow transplant (BMT) outcome but the results are inconsistent. As each of these studies examined single SNPs, the conflict could be due to different linkages between these SNPs and other functional SNPs and the corresponding phenotypes. A more comprehensive study of diversity and SNP linkages was undertaken here. Ten novel polymorphisms and 14 novel alleles were identified by sequence characterization of 38 unrelated individuals. The TGF-β1 alleles clustered into three phylogenetic groups based on the common functional SNPs −509C-T and +869T-C suggesting three phenotypic groups. However, the −509 and +869 SNP positions might not be as informative for predicting TGF-β1 phenotypes as suggested by the allelic groups. For example, individuals who carry allele p014 (intermediate phenotype) are more likely to have a low production phenotype due to the presence of +915C (decreased TGF-β1 expression) in this allele. This observation highlights why limited genotyping to predict phenotypes may not be definitive as linked SNPs likely affect the expected phenotypes that would be attributed to single SNPs. To assess impact of TGF-β1 promoter genotype on likelihood of developing and/or severity of graft-versus-host-disease (GVHD) in bone marrow transplant patients, we are characterizing 40 unrelated donor/recipient pairs in a pilot study. The genotype, p001/p003, was frequent (12/17 70.5%) in recipients with grade 3 and 4 GVHD in comparison to recipients with GVHD grades 0-2 (7/16 43.7%). These data target certain TGF-β1 promoter alleles for further study.

172

THE UNEXPECTED AND DIFFERENTIAL EFFECT OF CYCLOSPORIN A ON CD56^{bright} AND CD56^{dim} NK CELL EXPANSION, PHENOTYPE, FUNCTION AND DEVELOPMENT FROM PROGENITOR CELLS

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Following allo-HCT, NK cells play important roles in engraftment, anti-viral responses, GVHD and GVL. Cyclosporine A (CsA), is frequently administered to prevent or treat GVHD. It is generally considered that CsA inhibits GVL. To date, little is known about the impact of CsA on NK cell function. To investigate this, NK cells (from healthy donors) were cultured with IL-2, IL-15 and either physiological levels of CsA (1 μg/ml) or vehicle control. CsA treated cultures showed reduced NK cell expansion at one week (4.88 vs. 1.87 fold expansion, n = 10). The phenotype of CsA treated NK cells was markedly different than controls. After 7-10 days of culture with CsA there were significantly more CD56^{bright}CD16^{-} cells and significantly less CD56^{dim}CD16^{+} cells ($P < .001$ for both). The percentage of KIR receptor (CD158a/h, CD158b/j and CD158e) expressing cells was significantly less in CsA treated cultures. No changes were detected in NKG2D, NKp30, NKp44, or NKp46. To further investigate the influence of CsA on NK cell subset expansion, freshly isolated NK cells were stained with CFSE then cultured with CsA or vehicle control for 1 week. Using FACS we monitored CD56^{dim}CD16^{+} and CD56^{bright}CD16^{-} cell division. There was no difference in the proliferation of CD56^{bright}CD16^{-} cells between the CsA and control treated cultures. In contrast, the CsA treated CD56^{dim}CD16^{+} cells had fewer cell divisions, demonstrating that CsA selectively inhibits CD56^{dim}CD16^{+} cell proliferation. To investigate the cytotoxicity of CsA treated NK cells, we performed killing assays. Surprisingly, we found higher cytotoxicity in CsA treated NK cells compared to controls (K562, $P < .05$ and Raji, $P < .05$). To further evaluate CsA treated NK cells, we investigated the intracellular IFN-γ secretion following IL-12/IL-18 stimulation. In 5 consecutive experiments the percentage of IFN-γ secreting cells was higher in CsA treated NK cells (44% vs. 24%, $P < .05$). Lastly, we determined the effect of CsA on NK cell differentiation from progenitor cells (CD34^{+}Lin^{-}CD38^{-}) using an in vitro differentiation system. Briefly, progenitor cells were cultured on a murine feeder cell line (AFT-024) for 42 days in the presence of IL-3, IL-7, IL-15, SCF and FLT3L ± CsA. CsA treated cultures had less KIR expressing cells compared to controls. Collectively these results show that physiological levels of CsA results in NK cells that have less KIR receptors, higher cytotoxicity and more cytokine secretion.